EN Molecular Detection Assay 2 - *Salmonella*

2

Salmonella

Issue Date: 2015-10

3M

Product Instructions

Molecular Detection Assay 2 - *Salmonella* MDA2SAL96

PRODUCT DESCRIPTION AND INTENDED USE

The 3M™ Molecular Detection Assay 2 - *Salmonella* is used with the 3M™ Molecular Detection System for the rapid and specific detection of *Salmonella* in enriched food, feed and food process environmental samples.

The 3M Molecular Detection Assays use loop-mediated isothermal amplification to rapidly amplify nucleic acid sequences with high specificity and sensitivity, combined with bioluminescence to detect the amplification. Presumptive positive results are reported in real-time while negative results are displayed after the assay is completed. Presumptive positive results should be confirmed using your preferred method or as specified by local regulations [1, 2, 3].

The 3M Molecular Detection Assay 2 - *Salmonella* is intended for use in a laboratory environment by professionals trained in laboratory techniques. 3M has not documented the use of this product in industries other than food or beverage. For example, 3M has not documented this product for testing pharmaceutical, cosmetics, clinical or veterinary samples. The 3M Molecular Detection Assay 2 - *Salmonella* has not been evaluated with all possible food products, food processes, testing protocols or with all possible strains of bacteria.

As with all test methods, the source, formulation and quality of enrichment medium can influence the results. Factors such as sampling methods, testing protocols, sample preparation, handling, and laboratory technique may also influence results. 3M recommends evaluation of the method including enrichment medium, in the user's environment using a sufficient number of samples with particular foods and microbial challenges to ensure that the method meets the user's criteria.

3M has evaluated the 3M Molecular Detection Assay 2 - *Salmonella* with Buffered Peptone Water ISO.

The 3M™ Molecular Detection Instrument is intended for use with samples that have undergone heat treatment during the assay lysis step, which is designed to destroy organisms present in the sample. Samples that have not been properly heat treated during the assay lysis step may be considered a potential biohazard and should NOT be inserted into the 3M Molecular Detection Instrument.

3M Food Safety is certified to ISO (International Organization for Standardization) 9001 for design and manufacturing.

The 3M Molecular Detection Assay 2 - *Salmonella* test kit contains 96 tests, described in Table 1.

Table 1. Kit Components

Item	Identification	Quantity	Contents	Comments
Lysis Solution (LS) tubes	Pink solution in clear tubes	96 (12 strips of 8 tubes)	580 µL of LS per tube	Racked and ready to use
Salmonella Reagent tubes	Green tubes	96 (12 strips of 8 tubes)	Lyophilized specific amplification and detection mix	Ready to use
Extra caps	Green caps	96 (12 strips of 8 caps)		Ready to use
Reagent Control (RC)	Clear flip-top tubes	16 (2 pouches of 8 individual tubes)	Lyophilized control DNA, amplification and detection mix	Ready to use
Quick Start Guide		1		

The Negative Control, not provided in the kit, is a sterile enrichment medium, e.g., BPW ISO. Do not use water as a Negative Control.

SAFETY

The user should read, understand and follow all safety information in the instructions for the 3M Molecular Detection System and the 3M Molecular Detection Assay 2 - *Salmonella*. Retain the safety instructions for future reference.

⚠ **WARNING:** Indicates a hazardous situation, which, if not avoided, could result in death or serious injury and/or property damage.

⚠ **CAUTION:** Indicates a hazardous situation, which, if not avoided, could result in minor or moderate injury and/or property damage.

NOTICE: Indicates a potentially hazardous situation which, if not avoided, could result in property damage.

⚠ WARNING

Do not use the 3M Molecular Detection Assay 2 - *Salmonella* in the diagnosis of conditions in humans or animals.

The user must train its personnel in current proper testing techniques: for example, Good Laboratory Practices, ISO/IEC 17025[4], or ISO 7218[5].

To reduce the risks associated with a false-negative result leading to the release of contaminated product:

- Follow the protocol and perform the tests exactly as stated in the product instructions.
- Store the 3M Molecular Detection Assay 2 - *Salmonella* as indicated on the package and in the product instructions.
- Always use the 3M Molecular Detection Assay 2 - *Salmonella* by the expiration date.
- Use the 3M Molecular Detection Assay 2 - *Salmonella* with food, feed and food process environmental samples that have been validated internally or by a third party.

- Use the 3M Molecular Detection Assay 2 - *Salmonella* only with surfaces, sanitizers, protocols and bacterial strains that have been validated internally or by a third party.
- For an environmental sample containing Neutralizing Buffer with aryl sulfonate complex, perform a 1:2 dilution before testing (1 part sample into 1 part sterile enrichment broth). Another option is to transfer 10 µL of the NB enrichment into the LS tubes. 3M™ sample handling products which include Neutralizing Buffer with aryl sulfonate complex: BPPFV10NB, RS96010NB, RS9604NB, SSL10NB, XSLSSL10NB, HS10NB and HS119510NB.

To reduce the risks associated with exposure to chemicals and biohazards:

- Perform pathogen testing in a properly equipped laboratory under the control of trained personnel.
- Always follow standard laboratory safety practices, including wearing appropriate protective apparel and eye protection while handling reagents and contaminated samples.
- Avoid contact with the contents of the enrichment media and reagent tubes after amplification.
- Dispose of enriched samples according to current industry standards.

To reduce the risks associated with cross-contamination while preparing the assay:

- Always wear gloves (to protect the user and prevent introduction of nucleases).

To reduce the risks associated with environmental contamination:

- Follow current industry standards for disposal of contaminated waste.

⚠ CAUTION

- Do not exceed the recommended temperature setting on heater.
- Do not exceed the recommended heating time.
- Use an appropriate, calibrated thermometer to verify the 3M™ Molecular Detection Heat Block Insert temperature (e.g., a partial immersion thermometer or digital thermocouple thermometer, not a total immersion thermometer). The thermometer must be placed in the designated location in the 3M Molecular Detection Heat Block Insert.

NOTICE

To reduce the risks associated with cross-contamination while preparing the assay:

- Use of sterile, aerosol barrier (filtered), molecular biology grade pipette tips is recommended.
- Use a new pipette tip for each sample transfer.
- Use Good Laboratory Practices to transfer the sample from the enrichment to the lysis tube. To avoid pipettor contamination, the user may choose to add an intermediate transfer step. For example, the user can transfer each enriched sample into a sterile tube.
- Use a molecular biology workstation containing germicidal lamp where available.

To reduce the risks associated with a false-positive result:

- Never open tubes post amplification.
- Always dispose of the contaminated tubes by soaking in a 1-5% (v:v in water) household bleach solution for 1 hour and away from the assay preparation area.

Consult the Safety Data Sheet for additional information and local regulations for disposal.

If you have questions about specific applications or procedures, please visit our website at www.3M.com/foodsafety or contact your local 3M representative or distributor.

USER RESPONSIBILITY

Users are responsible for familiarizing themselves with product instructions and information. Visit our website at **www.3M.com/foodsafety**, or contact your local 3M representative or distributor for more information.

When selecting a test method, it is important to recognize that external factors such as sampling methods, testing protocols, sample preparation, handling, and laboratory technique may influence results.

It is the user's responsibility in selecting any test method or product to evaluate a sufficient number of samples with the appropriate matrices and microbial challenges to satisfy the user that the chosen test method meets the user's criteria.

It is also the user's responsibility to determine that any test methods and results meet its customers' and suppliers' requirements.

As with any test method, results obtained from use of any 3M Food Safety product do not constitute a guarantee of the quality of the matrices or processes tested.

To help customers evaluate the method for various food matrices, 3M has developed the 3M™ Molecular Detection Matrix Control kit. When needed, use the Matrix Control (MC) to determine if the matrix has the ability to impact the 3M Molecular Detection Assay 2 - *Salmonella* results. Test several samples, representative of the matrix, i.e. samples obtained from different origin, during any validation period when adopting the 3M method or when testing new or unknown matrices or matrices that have undergone raw material or process changes.

A matrix can be defined as a type of product with intrinsic properties such as composition and process. Differences between matrices may be as simple as the effects caused by differences in their processing or presentation for example, raw vs. pasteurized; fresh vs. dried, etc.

LIMITATION OF WARRANTIES / LIMITED REMEDY

EXCEPT AS EXPRESSLY STATED IN A LIMITED WARRANTY SECTION OF INDIVIDUAL PRODUCT PACKAGING, 3M DISCLAIMS ALL EXPRESS AND IMPLIED WARRANTIES, INCLUDING BUT NOT LIMITED TO, ANY WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR USE. If any 3M Food Safety Product is defective, 3M or its authorized distributor will, at its option, replace or refund the purchase price of the product. These are your exclusive remedies. You must promptly notify 3M within sixty days of discovery of any suspected defects in a product and return it to 3M. Please call Customer Service (1-800-328-1671 in the U.S.) or your official 3M Food Safety representative for a Returned Goods Authorization.

LIMITATION OF 3M LIABILITY

3M WILL NOT BE LIABLE FOR ANY LOSS OR DAMAGES, WHETHER DIRECT, INDIRECT, SPECIAL, INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING BUT NOT LIMITED TO LOST PROFITS. In no event shall 3M's liability under any legal theory exceed the purchase price of the product alleged to be defective.

STORAGE AND DISPOSAL

Store the 3M Molecular Detection Assay 2 - *Salmonella* at 2-8°C. Do not freeze. Keep kit away from light during storage. After opening the kit, check that the foil pouch is undamaged. If the pouch is damaged, do not use. After opening, unused reagent tubes should always be stored in the re-sealable pouch with the desiccant inside to maintain stability of the lyophilized reagents. Store resealed pouches at 2-8°C for no longer than 60 days.

Do not use 3M Molecular Detection Assay 2 - *Salmonella* past the expiration date. Expiration date and lot number are noted on the outside label of the box. After use, the enrichment medium and the 3M Molecular Detection Assay 2 - *Salmonella* tubes can potentially contain pathogenic materials. When testing is complete, follow current industry standards for the disposal of contaminated waste. Consult the Safety Data Sheet for additional information and local regulations for disposal.

INSTRUCTIONS FOR USE

Follow all instructions carefully. Failure to do so may lead to inaccurate results.

Periodically decontaminate laboratory benches and equipment (pipettes, cap/decap tools, etc.) with a 1- 5% (v:v in water) household bleach solution or DNA removal solution.

SAMPLE ENRICHMENT

Table 2 presents guidance for general enrichment protocols for food, feed and environmental samples.

It is the user's responsibility to validate alternate sampling protocols or dilution ratios to ensure this test method meets the user's criteria.

Foods

1. Allow BPW ISO enrichment medium to equilibrate to ambient laboratory temperature or 41.5 ±1°C depending upon matrices tested. See Tables 2 and 3.
2. Aseptically combine the enrichment medium and sample. For all meat and highly particulate samples, the use of filter bags is recommended.
3. Homogenize thoroughly by blending, stomaching, or hand mixing for 2 ±0.2 minutes. Incubate as outlined in the appropriate protocol table (See Tables 2 and 3).

Environmental samples

Sample collection devices can be a sponge hydrated with a neutralizing solution to inactivate the effects of the sanitizers. 3M recommends the use of a biocide-free cellulose sponge. Neutralizing solution can be Dey-Engley (D/E) Neutralizing Broth or Letheen Broth. It is recommended to sanitize the area after sampling.

WARNING: Should you select to use Neutralizing Buffer (NB) that contains aryl sulfonate complex as the hydrating solution for the sponge, it is required to perform a 1:2 dilution (1 part sample into 1 part sterile enrichment broth) of the enriched environmental sample before testing in order to reduce the risks associated with a false-negative result leading to the release of contaminated product. Another option is to transfer 10 µL of the NB enrichment into the LS tubes.

The recommended size of the sampling area to verify the presence or absence of the pathogen on the surface is at least 100 cm^2 (10 cm x 10 cm or 4"x4"). When sampling with a sponge, cover the entire area going in two directions (left to right then up and down) or collect environmental samples following your current sampling protocol or according to the FDA BAM[1], USDA FSIS MLG[2] or ISO 18593:2004[8] guidelines.

It is the user's responsibility to validate alternate sampling protocols or dilution ratios to ensure this test method meets the user's criteria.

1. Pre-warm BPW ISO enrichment medium to 41.5 ±1°C depending upon matrices tested. See Tables 2 and 3.
2. Aseptically combine the enrichment medium and sample. Homogenize thoroughly by blending, stomaching, or hand mixing for 2 ±0.2 minutes. Incubate as outlined in the appropriate protocol table (See Tables 2 and 3).

Table 2. General enrichment protocols

Sample Matrix	Sample Size	Enrichment Broth Volume	Enrichment Temperature (±1°C)	Enrichment Time (hr)	Sample Analysis Volume[a]
Protocol 1 Processed food products (excluding egg powders, and products specified in the other protocols)[b]	25 g	225 mL BPW ISO	37	18-26	20 µL
Protocol 2 Raw unprocessed frozen food, egg powders, animal feed, and environmental samples[c]	25 g	225 mL BPW ISO (pre-warmed)	41.5	18-26	20 µL
Protocol 3 Powdered dairy products (includes infant formula, soy based infant formula)	25 g	225 mL BPW ISO	37	20-26	20 µL
Protocol 4 Cocoa based products (powder, chocolates, confectionaries, etc.)	25 g	225 mL 100 g/L non-fat dry milk with 0.002% brilliant green dye[d,e]	37	24-30	20 µL
Protocol 5 Other including: spices, aromatic herbs, concentrates, instant teas and coffees, bouillon cubes	25 g	235 mL 2X BPW ISO with 0.5% K_2SO_3 + 240 mL 100 g/L nonfat dry milk[f,g]	37	24-30	10 µL

(a) Volume of sample transferred to Lysis Solution tubes. Refer to step 4.6 of Lysis section.
(b) Examples of products to be tested with Protocol 1: ready to use meals, deli salads, custard.
(c) Examples of products to be tested with Protocol 2: raw meats, frozen vegetables, all cheeses, fermented milk, raw salad (lettuce, Batavia).
(d) Non-fat UHT milk can be substituted for non-fat dry milk.
(e) 0.45 mL of 1% aqueous brilliant green dye solution per 225 mL non-fat milk, resulting in a final concentration of 0.002% (0.02 g/L) brilliant green dye.
(f) 5 g of K_2SO_3 per 1000 mL BPW ISO, resulting in a final concentration of 0.5% K_2SO_3.
(g) 240 mL 100 g/L sterile non-fat dry milk must be added to sterilized 235 mL 2X BPW ISO with 0.5% K_2SO_3.
(h) If using an optional secondary enrichment step, e.g. Rappaport Vassiliadis Medium, it is required to perform a 1:2 dilution (1 part sample enrichment into 1 part sterile enrichment broth) or simply transfer 10 µL of the secondary enrichment to the LS tubes. If using TT Broth, do not vortex to avoid transfer of any precipitate.

Specific Instructions for Validated Methods
AOAC® Performance Tested Method℠ #091501

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 091501

In AOAC PTM studies, the 3M Molecular Detection Assay 2 - *Salmonella* was found to be an effective method for the detection of *Salmonella*. The matrices tested in the study are shown in Table 3.

Table 3. Enrichment Protocols according to AOAC PTM #091501.

Volume of sample transferred to Lysis Solution (LS) tubes is 20 µL.

Sample Matrix		Sample Size	Enrichment Broth Volume	Enrichment Temperature (±1°C)	Enrichment Time (hr)
Raw ground beef		25 g	225 mL BPW ISO (pre-warmed)	41.5	10-24
		325 g	975 mL BPW ISO (pre-warmed)		
Raw ground chicken		25 g	225 mL BPW ISO (pre-warmed)	41.5	10-24
		325 g	975 mL BPW ISO (pre-warmed)	41.5	14-24
Cooked breaded chicken		325 g	2,925 mL BPW ISO	37	18-24
Dry dog food		25 g	225 mL BPW ISO	37	18-24
		375 g	1,500 mL BPW ISO		
Black pepper, Raw whole shrimp, Raw bagged spinach, Pasteurized processed American cheese		25 g	225 mL BPW ISO	37	18-24
Chicken carcass rinse		30 mL	30 mL BPW ISO (pre-warmed)	41.5	18-24
Chicken carcass sponge		1 sponge	50 mL BPW ISO (pre-warmed)	41.5	18-24
Instant non-fat dry milk		25g	225 mL BPW ISO	37	20-24
Cocoa powder		25g	225 mL BPW ISO	37	24-28
Pasteurized liquid whole egg		100 mL	900 mL BPW ISO	37	18-24
Spent sprout irrigation water		375 mL	3,375 mL BPW ISO	37	18-24
Creamy peanut butter		25 g	225 mL BPW ISO	37	18-24
		375 g	3,375 mL BPW ISO		
Environmental	Sealed concrete	1 sponge	225 mL BPW ISO (pre-warmed)	41.5	18-24
	Stainless steel	1 swab	10 mL BPW ISO (pre-warmed)	41.5	18-24
	Sealed ceramic tile	1 sponge	50 mL BPW ISO (pre-warmed)	41.5	18-24

NOTES:
The recommended protocol interruption points are after enrichment or after sample lysis. Enrichment broth or sample lysate can be stored at 2-8°C for up to 72 hours. After removing the enrichment broth from storage, resume testing from Step 1 in the **LYSIS** section. After removing the sample lysate from storage, resume testing from Step 7 in the **LYSIS** section.

PREPARATION OF THE 3M™ MOLECULAR DETECTION SPEED LOADER TRAY

1. Wet a cloth or disposable towel with a 1-5% (v:v in water) household bleach solution and wipe the 3M™ Molecular Detection Speed Loader Tray.
2. Rinse the 3M Molecular Detection Speed Loader Tray with water.
3. Use a disposable towel to wipe the 3M Molecular Detection Speed Loader Tray dry.
4. Ensure the 3M Molecular Detection Speed Loader Tray is dry before use.

PREPARATION OF THE 3M™ MOLECULAR DETECTION CHILL BLOCK INSERT

Place the 3M™ Molecular Detection Chill Block Insert directly on the laboratory bench: The 3M Molecular Detection Chill Block Tray is not used. Use the block at ambient laboratory temperature (20-25°C).

PREPARATION OF THE 3M™ MOLECULAR DETECTION HEAT BLOCK INSERT

Place the 3M™ Molecular Detection Heat Block Insert in a dry double block heater unit. Turn on the dry block heater unit and set the temperature to allow the 3M Molecular Detection Heat Block Insert to reach and maintain a temperature of 100 ±1°C.

NOTE: Depending on the heater unit, allow approximately 30 minutes for the 3M Molecular Detection Heat Block Insert to reach temperature. Using an appropriate, calibrated thermometer (e.g., a partial immersion thermometer, digital thermocouple thermometer, not a total immersion thermometer) placed in the designated location, verify that the 3M Molecular Detection Heat Block Insert is at 100 ±1°C.

PREPARATION OF THE 3M MOLECULAR DETECTION INSTRUMENT

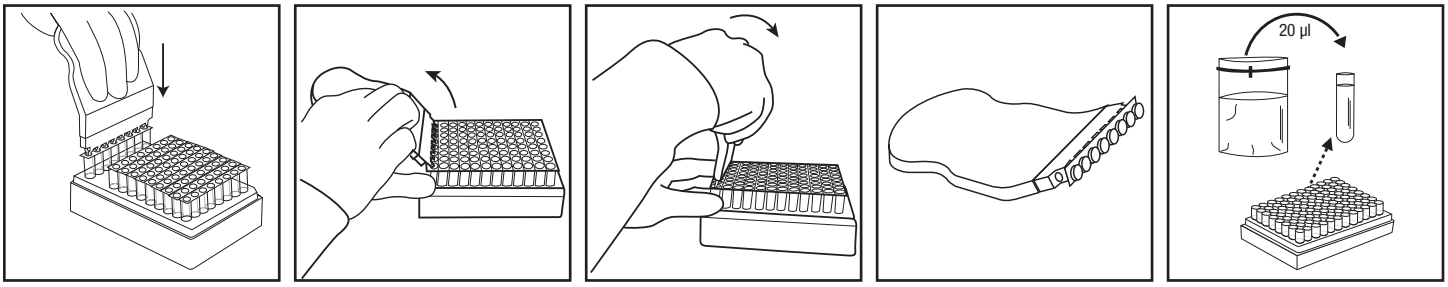
1. Launch the 3M™ Molecular Detection Software and log in.
2. Turn on the 3M Molecular Detection Instrument.
3. Create or edit a run with data for each sample. Refer to the 3M Molecular Detection System User Manual for details.

NOTE: The 3M Molecular Detection Instrument must reach and maintain temperature of 60°C before inserting the 3M Molecular Detection Speed Loader Tray with reaction tubes. This heating step takes approximately 20 minutes and is indicated by an ORANGE light on the instrument's status bar. When the instrument is ready to start a run, the status bar will turn GREEN.

LYSIS

1. Allow the lysis solution (LS) tubes to warm up by setting the rack at room temperature (20-25°C) overnight (16-18 hours). Alternatives to equilibrate the LS tubes to room temperature are to set the LS tubes on the laboratory bench for at least 2 hours, incubate the LS tubes in a 37 ±1°C incubator for 1 hour or place them in a dry double block heater for 30 seconds at 100°C.
2. Invert the capped tubes to mix. Proceed to next step within 4 hours.
3. Remove the enrichment broth from the incubator.
4. One LS tube is required for each sample and the Negative Control (NC) sample (sterile enrichment medium).
   - 4.1 LS tube strips can be cut to desired LS tube number. Select the number of individual LS tubes or 8-tube strips needed. Place the LS tubes in an empty rack.
   - 4.2.To avoid cross-contamination, decap one LS tubes strip at a time and use a new pipette tip for each transfer step.
   - 4.3.Transfer enriched sample to LS tubes as described below:

     Transfer each enriched sample into an individual LS tube **first**. Transfer the NC **last**.
   - 4.4 Use the 3M™ Molecular Detection Cap/Decap Tool-Lysis to decap one LS tube strip - one strip at a time.
   - 4.5 Discard the LS tube cap – If lysate will be retained for retest, place the caps into a clean container for re-application after lysis.
     - 4.5.1 For processing of retained lysate, see Appendix A.
   - 4.6 Transfer 20 µL of sample into a LS tube unless otherwise indicated in the protocol table (example protocol 5).
5. Repeat step 4.2 until each individual sample has been added to a corresponding LS tube in the strip.



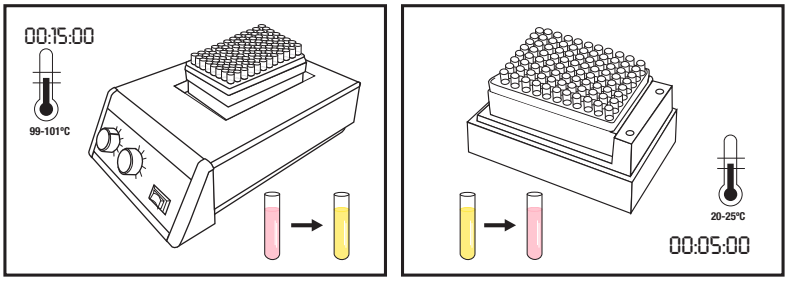
6. Repeat steps 4.1 to 4.6 as needed, for the number of samples to be tested.
7. When all samples have been transferred, transfer 20 µL of NC (sterile enrichment medium e.g. BPW) into a LS tube. Do not use water as a NC.
8. Verify that the temperature of the 3M Molecular Detection Heat Block Insert is at 100 ±1°C.

9. Place the uncovered rack of LS tubes in the 3M Molecular Detection Heat Block Insert and heat for 15 ±1 minutes. During heating, the LS solution will change from pink (cool) to yellow (hot).

   Samples that have not been properly heat treated during the assay lysis step may be considered a potential biohazard and should NOT be inserted into the 3M Molecular Detection Instrument.

10. Remove the uncovered rack of LS tubes from the heating block and allow to cool in the 3M Molecular Detection Chill Block Insert at least 5 minutes and a maximum of 10 minutes. The 3M Molecular Chill block Insert, used at ambient temperature without the Molecular Detection Chill Block Tray, should sit directly on the laboratory bench. When cool, the lysis solution will revert to a pink color.

11. Remove the rack of LS tubes from the 3M Molecular Detection Chill Block Insert.



AMPLIFICATION

1. One Reagent tube is required for each sample and the NC.

   1.1 Reagent tube strips can be cut to desired tube number. Select the number of individual Reagent tubes or 8-tube strips needed.

   1.2 Place Reagent tubes in an empty rack.

   1.3 Avoid disturbing the reagent pellets from the bottom of the tubes.

2. Select 1 Reagent Control (RC) tube and place in rack.
3. To avoid cross-contamination, decap one Reagent tube strip at a time and use a new pipette tip for each transfer step.
4. Transfer lysate to Reagent tubes and RC tube as described below:

Transfer each sample lysate into individual Reagent tubes **first** followed by the NC. Hydrate the RC tube **last**.

5. Use the 3M™ Molecular Detection Cap/Decap Tool-Reagent to decap the Reagent tubes –one Reagent tube strip at a time. Discard cap.

   5.1 Transfer 20 µL of Sample lysate from the upper ½ of the liquid (avoid precipitate) in the LS tube into corresponding Reagent tube. Dispense at an angle to avoid disturbing the pellets. Mix by gently pipetting up and down 5 times.

   5.2 Repeat step 4.2 until individual Sample lysate has been added to a corresponding Reagent tube in the strip.

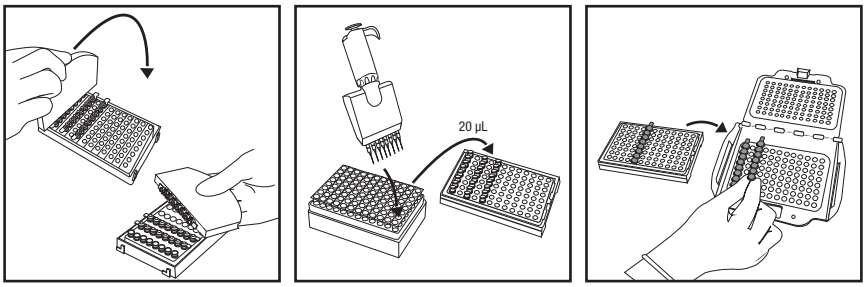
   5.3 Cover the Reagent tubes with the provided extra caps and use the rounded side of the 3M Molecular Detection Cap/Decap Tool-Reagent to apply pressure in a back and forth motion ensuring that the cap is tightly applied.

   5.4 Repeat steps 4.1 to 4.4 as needed, for the number of samples to be tested.

   5.5 When all sample lysates have been transferred, repeat 4.1 to 4.4 to transfer 20 µL of NC lysate into a Reagent tube.

   5.6 Transfer **20 µL of NC lysate into a RC tube.** Dispense at an angle to avoid disturbing the pellets. Mix by gently pipetting up and down 5 times.

6. Load capped tubes into a clean and decontaminated 3M Molecular Detection Speed Loader Tray. Close and latch the 3M Molecular Detection Speed Loader Tray lid.



7. Review and confirm the configured run in the 3M Molecular Detection Software.
8. Click the Start button in the software and select instrument for use. The selected instrument's lid automatically opens.
9. Place the 3M Molecular Detection Speed Loader Tray into the 3M Molecular Detection Instrument and close the lid to start the assay. Results are provided within 75 minutes, although positives may be detected sooner.

10. After the assay is complete, remove the 3M Molecular Detection Speed Loader Tray from the 3M Molecular Detection Instrument and dispose of the tubes by soaking in a 1-5% (v:v in water) household bleach solution for 1 hour and away from the assay preparation area.

NOTICE: To minimize the risk of false positives due to cross-contamination, never open reagent tubes containing amplified DNA. This includes Reagent Control, Reagent, and Matrix Control tubes. Always dispose of sealed reagent tubes by soaking in a 1-5% (v:v in water) household bleach solution for 1 hour and away from the assay preparation area.

RESULTS AND INTERPRETATION

An algorithm interprets the light output curve resulting from the detection of the nucleic acid amplification. Results are analysed automatically by the software and are color-coded based on the result. A Positive or Negative result is determined by analysis of a number of unique curve parameters. Presumptive positive results are reported in real-time while Negative and Inspect results will be displayed after the run is completed.

Presumptive positive samples should be confirmed as per the laboratory standard operating procedures or by following the appropriate reference method confirmation[1,2,3], beginning with transfer from the primary 3M BPW ISO enrichment to secondary enrichment broth(s), followed by subsequent plating and confirmation of isolates using appropriate biochemical and serological methods.

NOTE: Even a negative sample will not give a zero reading as the system and 3M Molecular Detection Assay 2 - *Salmonella* amplification reagents have a "background" relative light unit (RLU) reading.

In the rare event of any unusual light output, the algorithm labels this as "Inspect." 3M recommends the user to repeat the assay for any Inspect samples. If the result continues to be Inspect, proceed to confirmation test using your preferred method or as specified by local regulations.

In the event of discordant results (presumptive positive with the 3M Molecular Detection Assay 2 - *Salmonella*, non-confirmed by one of the means described above, and in particular for the latex agglutination test), the laboratory must follow the necessary steps to ensure the validity of the results obtained.

If you have questions about specific applications or procedures, please visit our website at www.3M.com/foodsafety or contact your local 3M representative or distributor.

REFERENCES:

1. US Food and Drug Administration Bacteriological Analytical Manual. Chapter 5: *Salmonella*, Section C-24. November 2011 Version.
2. US Department of Agriculture (USDA) FSIS Microbiology Laboratory Guidebook 4.05. Isolation and Identification of *Salmonella* from Meat, Poultry, Pasteurized Egg, and Catfish Products. Effective Date: 20 January 2011.
3. ISO 6579. Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp.
4. ISO/IEC 17025. General requirements for the competence of testing and calibration laboratories.
5. ISO 7218. Microbiology of food and animal feeding stuffs – General rules for microbiological examination.
6. ISO 16140. Microbiology of food and animal feeding stuffs – Protocol for the validation of alternative methods.
7. ISO 6887. Microbiology of food and animal feeding stuffs – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination.
8. ISO 18593. Microbiology of food and animal feeding stuffs – Horizontal methods for sampling techniques from surfaces using contact plates and swabs.

Appendix A. Protocol Interruption: Storage and re-testing of heat-treated lysates

1. To store a heat-treated lysate, re-cap the lysis tube with a clean cap (see "LYSIS", 4.5)
2. Store at 4 to 8°C for up to 72 hours.
3. Prepare a stored sample for amplification by inverting 2-3 times to mix.
4. Decap the tubes.
5. Place the mixed lysate tubes on 3M Molecular Detection Heat Block Insert and heat at 100 ±1°C for 5 ±1 minutes.
6. Remove the rack of LS tubes from the heating block and allow to cool in the 3M Molecular Detection Chill Block Insert at least 5 minutes and a maximum of 10 minutes.
7. Continue the protocol at the 'Amplification' section detailed above.

EXPLANATION OF PRODUCT LABEL SYMBOLS

 Caution or Warning, see product instructions.

 Consult product instructions.

 Batch code.

 Use by date.

 Store between given temperatures.

3M Food Safety

3M United States
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Bldg. 275-5W-05
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1-800-328-6553

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1-954-340-8263

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65-64508869

3M Japan
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81-570-011-321

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34-8717-9389-8